

marker, and said method further comprises selecting said transformants with said second selection marker.

66. (New) The method according to claim 65 wherein said first selection marker and said second selection marker are different antibiotic resistance genes.--

REMARKS

Reconsideration is requested.

Claims 47-57 of the Amendment of October 2, 2002 have been canceled above, without prejudice.¹

Claims 58-66 have been added. The Examiner indicated in Paper No. 22, that amending claims 47 and 55 to include the details of claims 49 and 57, respectively, "would appear" to overcome the Section 112, first paragraph, "written description" rejection of claims 47-57.² Claim 58 above combines the details of canceled claims 47 and 49 and claim 60 above combines the details of canceled claims 50 and 57, without prejudice, to advance prosecution. Entry of the above amendments will, at a minimum,

¹ The undersigned has assumed the Amendment of October 2, 2002, has been entered, as indicated in the Advisory Action of October 29, 2002 (Paper No. 22). The new claims, presented above, therefore have been numbered from claims 58. The Examiner is requested to advise the undersigned if the new claims should be otherwise numbered.

² While the Examiner indicates the details of claims 55 and 57 could be combined to overcome the rejection, the undersigned believes the Examiner perhaps meant to indicate that the details of claims 50 and 57 should be combined to overcome the rejection as claim 55 is dependent on the independent claim 50. The above amendments are based on the undersigned's assumption and the Examiner is requested to contact the undersigned in the event the Examiner's comments have been misunderstood.

reduce the issues for appeal by obviating the Section 112, first paragraph, "written description" rejection of the claims. Entry of the amendments is requested.

The above amendments, as well as the amendments of October 2, 2002, are believed to obviate the Section 112, first paragraph, "enablement" rejection of the claims, in that the Examiner indicated in the Office Action of July 2, 2002 (Paper No. 19) that

"The specification is enabling for a recombinant cell comprising a polynucleotide encoding a Luc that is thermostable at temperatures of 37⁰C or more and a polynucleotide encoding a mutant AK polypeptide that is inactivated at temperatures of 37⁰C or more, wherein the Luc maintains at least partial enzymatic activity and methods of making said recombinant cell or using said recombinant cell for the production of Luc." See, page 5 of Paper No. 19.

The claims were amended in the Amendment of October 2, 2002 and above in response to these comments by the Examiner, to advance prosecution. The applicants inadvertence in not specifically noting the same in the remarks of the Amendment of October 2, 2002, as noted by the Examiner in Paper No.22, is unfortunate however the claims are believed to obviate the Section 112, first paragraph "enablement" rejection. Entry of the above will therefore, at a minimum, obviate the Section 112, first paragraph, "enablement" rejection. Entry of the amendments and withdrawal of the rejection are requested.

The Section 103 rejection of claims 47-57 over EP 373962, Belinga (J. Chrom A 695:33-40), Gilles (PNAS 83:5798-5802) and Kajiyama (Biochemistry 32:13795-13799) is traversed. Reconsideration and withdrawal of the rejection are requested.

The claims have been amended above in response to the Examiner's comment on page 4 of Paper No. 22. Specifically, the Examiner indicated the applicants previous

arguments relating to the use of temperatures as low as 37⁰C were not persuasive because the claims presented in the Amendment of October 2, 2002 allowed for inactivation a mutant adenylate kinase polypeptide at temperatures above 37⁰C. The independent claims 58 and 60 presented above recite adenylate kinase inactivation at 37⁰C, to advance prosecution and further define the claimed invention over the cited art.

The Examiner is again urged to appreciate that presently claimed invention is based on the discovery that in order to achieve a protein which is free from a particularly undesirable contaminant, one of ordinary skill in the art is able to engineer a host so that it not only expresses the target protein, but it also expresses the contaminant in an unstable form. The introduced instability can then be utilised to remove the particular contaminant. The method and host are particularly applicable to the production of luciferases where thermostability is known to be low, but has been improving of late as a result of new developing improvements and mutations.

None of the cited prior art teaches or suggests the claimed invention. Specifically, EP-A-373962 relates to expression of proteins, which have very high thermostability. The title alone (Isolating thermostable enzymes) teaches this. As EP-A-373962 teaches the production of thermostable enzymes, there is no motivation to produce the enzymes in an environment where the contaminants may be destroyed at the relatively low temperatures of the presently claimed invention. That is, there is no difficulty in removing possible contaminant proteins in the teaching of EP-A-373962, since the desired heterologous enzymes, being derived from thermophilic organisms such as *Thermus thermophilus*, are completely stable at very high temperatures, where all the

proteins of a "normal" expression cell would be denatured. Temperatures in excess of 70C are in fact used in EP-A-373962.

An ordinarily skilled person, attempting to produce a protein which is not generally regarded as being "thermostable" in the sense intended by EP-A-373962 would not consider this reference, as clearly it would not be applicable.

Kajiyama et al. teach a "thermostable luciferase". However, luciferase proteins are notoriously thermolabile, causing great problems for storage etc., even at room temperature. This is outlined by Kajiyama in column 1 lines 24-30 of the corresponding US Patent No. 5,229,285.

In order to address this problem, Kajiyama has engineered a mutated enzyme where the thermostability is better than that found in the wild-type enzyme. However, it is clearly still not a "thermostable" enzyme in the same way as the enzymes of EP-A-373962 are thermostable. The extent of the thermostability is set out in column 2 on page 13797 final paragraph, which makes it clear that enzyme activity reduced at 50C, albeit less dramatically than the wild-type enzyme. Further information regarding the actual thermostability of Kajiyama's mutant enzyme is given in the corresponding U.S. Patent No. 5,229,285. It is reported there, at in column 4, lines 62-69 that the protein is completely inactivated at 65C and that the enzyme activity is reduces over time when subjected to temperatures of 50C.

As a result, the applicants submit that an ordinarily skilled person would not have considered using the method of EP-A-373962 to produce a luciferase, even a purportedly thermostable luciferase such as that of Kajiyama. Hence the combination of EP-A-

373962 with Kajiyama fails to teach or suggest the presently claimed invention. An ordinarily skilled person, even if they read the two documents together, would not have been motivated to make the presently claimed invention.

The relatively low temperature inactivation of contaminants, such as is provided by the presently claimed invention, is not found in the cited art. There was no motivation in the art therefore to look to the cited Gilles and/or Belinga references. With due respect, the applicants again submit the Examiner has only found motivation to combine these teachings through an inappropriate use of hindsight.

The claims are submitted to be patentable over the art of record.

Entry of the above amendments and withdrawal of the Section 103 rejection are requested.

The claims, as amended, are submitted to be in condition for allowance and entry of the amendments and a Notice of Allowance are requested.

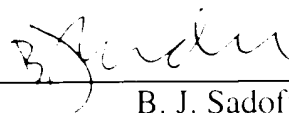
At a minimum, the applicants submit that entry of the amendments will obviate the Section 112, first paragraph, rejections, thus reducing the issues for appeal. Entry of the amendments is requested.

The Examiner is requested to contact the undersigned in the event anything further is required.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____



B. J. Sadoff
Reg. No. 36,663

SQUIRRELL et al.
Serial No. **09/529,722**

BJS:plb
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100